

What is claimed is:

1    1. A peptide comprising an amino acid sequence having a cleavage site specific for an  
2    enzyme having a proteolytic activity of prostate specific antigen, wherein the peptide is 20  
3    or fewer amino acids in length.

1    2. The peptide of claim 1, wherein the sequence comprises: the amino acids

3     $X_5X_4X_3X_2X_1$ ,

5    5    wherein  $X_5$  is from 0 to 16 amino acids;  $X_4$  is serine, isoleucine, or lysine;  $X_3$  is serine or  
6    lysine;  $X_2$  is leucine, tyrosine or lysine; and  $X_1$  is glutamine, asparagine or tyrosine.

1    3. The peptide of claim 2, further comprising  $X_{11}$  linked to  $X_1$ , wherein  $X_{11}$  is from 1 to  
2    10 amino acids.

1    4. The peptide of claim 2, wherein  $X_1$  is glutamine.

1    5. The peptide of claim 2, further comprising amino acid  $X_6$  linked to the amino terminus  
2    of  $X_5$ , wherein  $X_6$  is from 0 to 15 amino acids and wherein  $X_5$  is serine or lysine.

1    6. The peptide of claim 5, further comprising amino acid  $X_7$  linked to the amino terminus  
2    of  $X_6$ , wherein  $X_7$  is from 0 to 14 amino acids and wherein  $X_6$  is histidine or asparagine.

1    7. The peptide of claim 3, wherein  $X_{11}$  comprises leucine.

1    8. The peptide of claim 6, wherein the amino acid sequence is selected from the group  
2    consisting of His-Ser-Ser-Lys-Leu-Gln, Glu-His-Ser-Ser-Lys-Leu-Gln, Gln-Asn-Lys-Ile-  
3    /Ser-Tyr-Gln, and Glu-Asn-Lys-Ile-Ser-Tyr-Gln.

1 19. A composition comprising a prodrug, the prodrug comprising  
2 a therapeutically active drug; and  
3 a peptide of claim 1,  
4 wherein the peptide is linked to the therapeutically active drug to inhibit the  
5 therapeutic activity of the drug, and wherein the therapeutically active drug is cleaved  
6 from the peptide upon proteolysis by an enzyme having a proteolytic activity of prostate  
7 specific antigen (PSA).

1 20. The composition of claim 19, wherein the peptide is linked directly to the therapeutic  
2 drug.

1 21. The composition of claim 20, wherein the peptide is linked directly to a primary  
2 amine group on the drug.

1 22. The composition of claim 19, wherein the peptide is linked to the therapeutic drug via  
2 a linker.

1 23. The composition of claim 22, wherein the linker is an amino acid sequence.

1 24. The composition of claim 23, wherein the linker comprises a leucine residue.

1 25. The composition of claim 19, wherein the therapeutically active drug inhibits a  
2 SERCA pump.

1 26. The composition of claim 25, wherein the therapeutically active drug is selected from  
2 the group of primary amine containing thapsigargin or thapsigargin derivatives.

1 27. The composition of claim 19, wherein the therapeutically active drug intercalates into  
2 a polynucleotide.

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1 28. The composition of claim 27, wherein the therapeutically active drug is an  
2 anthracycline antibiotic.

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1 29. The composition of claim 28, wherein the therapeutically active drug is selected from  
2 the group consisting of doxorubicin, daunorubicin, epirubicin and idarubicin.

1 30. The composition of claim 19, wherein the peptide is His-Ser-Ser-Lys-Leu-Gln-Leu.

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1 31. The composition of claim 19, wherein the therapeutic drug is a compound belonging  
2 to the group of thapsigargins which have been derivatized with a moiety containing a  
3 primary amine group, the peptide is His-Ser-Ser-Lys-Leu-Gln, and the linker is selected  
4 from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido-  
5 or amino-substituted  $\text{CO}-(\text{CH}=\text{CH})_{n_1}-(\text{CH}_2)_{n_2}-\text{Ar}-\text{NH}_2$ ,  $\text{CO}-(\text{CH}_2)_{n_2}-(\text{CH}=\text{CH})_{n_1}-\text{Ar}-\text{NH}_2$ ,  
6  $\text{CO}-(\text{CH}_2)_{n_2}-(\text{CH}=\text{CH})_{n_1}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$  and  $\text{CO}-(\text{CH}=\text{CH})_{n_1}-(\text{CH}_2)_{n_2}\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$ ,  
7 wherein n1 and n2 are from 0 to 5, Ar is any substituted or unsubstituted aryl group, and  
8 attachment of  $\text{NH}_2$  to Ar is in a ortho, meta or para position with respect to the remainder  
9 of the linker.

13:  
1 32. The composition of claim 19, wherein the therapeutically active drug has an  $\text{IC}_{50}$   
2 toward ER  $\text{Ca}^{2+}$ -ATPase of at most 500 nM.

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15:  
1 33. The composition of claim 32, wherein the therapeutically active drug has an  $\text{IC}_{50}$   
2 toward ER  $\text{Ca}^{2+}$ -ATPase of at most 50 nM.

16:  
1 34. The composition of claim 19, wherein the therapeutically active drug has an  $\text{LC}_{50}$   
2 toward PSA-producing tissue of at most 20  $\mu\text{M}$ .

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17:  
1 35. The composition of claim 34, wherein the therapeutically active drug has an  $\text{LC}_{50}$   
2 toward PSA-producing tissue of less than or equal to 2.0  $\mu\text{M}$ .

1 16.36. The composition of claim 19, wherein cleavage of the peptide by the enzyme yields  
2 at least 5 picomoles of cleaved peptide per minute per 200 picomoles of enzyme.

1 A. 37. The composition of claim 19, wherein cleavage of the peptide in human serum yields  
2 at most 2.0 picomoles of cleaved peptide per minute.

1 D.

1 38. The composition of claim 19, further comprising an added substituent which renders  
2 the composition water soluble.

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1 39. The composition of claim 38, wherein the added substituent is a polysaccharide.

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1 40. The composition of claim 39, wherein the polysaccharide is selected from the group  
2 consisting of modified or unmodified dextran, cyclodextrin and starch.

1 41. A therapeutically active sesquiterpene- $\gamma$ -lactone derivative containing a primary  
2 amine.

1 42. The derivative of claim 41, wherein the sesquiterpene- $\gamma$ -lactone is a thapsigargin  
2 derivative.

1 43. The thapsagargin derivative of claim 42, further comprising a boc protecting group.

1 44. The thapsigargin derivative of claim 42, wherein the derivative is linked to an  
2 antibody.

*Sal-A2*

1 55. A method of producing a prodrug, the method comprising the step of linking  
2 a therapeutically active drug and  
3 a peptide of claim 1,  
4 wherein the linking of the peptide to the drug inhibits the therapeutic activity of  
5 the drug.

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1 56. The method of claim 55, wherein the therapeutically active drug has a primary amine.

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1 57. The method of claim 56, wherein the prodrug contains a linker between the peptide  
2 and the drug.

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1 58. The method of claim 57, wherein the linker comprises Leu.

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1 59. The method of claim 58, wherein the peptide further comprises a capping group  
2 attached to the N-terminus of the peptide, the group inhibiting endopeptidase activity on  
3 the peptide.

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1 60. The method of claim 59, wherein the capping group is selected from the group  
2 consisting of acetyl, morpholinocarbonyl, benzyloxycarbonyl, glutaryl, and succinyl  
3 substituents.

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1 61. A method of treating a PSA-producing cell proliferative disorder, the method  
2 comprising administering the composition of claim 19 in a therapeutically effective  
3 amount to a subject having the cell proliferative disorder.

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1 62. The method of claim 61, wherein the disorder is benign.

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1 63. The method of claim 61, wherein the disorder is malignant.

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1 64. The method of claim 63, wherein the malignant disorder is prostate cancer.

1        23              31  
1     65. The method of claim 63, wherein the malignant disorder is breast cancer.

1     66. A method of detecting prostate specific antigen-producing tissue, the method  
2       comprising:

3                 contacting the tissue with a composition comprising

4                 a detectably labeled peptide of claim 1 for a period of time sufficient to  
5                 allow cleavage of the peptide; and

6                 detecting the detectable label.

1     67. The method of claim 66, wherein the peptide further comprises a capping group  
2       attached to the N-terminus of the peptide, the group inhibiting endopeptidase activity.

1     68. The method of claim 67, wherein the capping group is selected from the group  
2       consisting of acetyl, morpholinocarbonyl, benzyloxycarbonyl, glutaryl, and succinyl  
3       substituents.

1     69. The method of claim 66, wherein the detectable label is a fluorescent label.

1     70. The method of claim 69, wherein the fluorescent label is selected from the group  
2       consisting of 7-amino-4-methyl coumarin, 7-amino-4-trifluoromethyl coumarin, rhodamine  
3       110, and 6-aminoquinoline.

1     71. The method of claim 66, wherein the detectable label is a radioactive label.

1     72. The method of claim 71, wherein the radioactive label is selected from the group  
2       consisting of tritium, carbon-14, and iodine-125.

1     73. The method of claim 66, wherein the detectable label is a chromophoric label.

1        9. The peptide of claim 1, further comprising a capping group attached to the N-terminus  
2        of the peptide, the group inhibiting endopeptidase activity on the peptide.

1        10. The peptide of claim 9, wherein the capping group is selected from the group  
2        consisting of acetyl, morpholinocarbonyl, benzyloxycarbonyl, glutaryl and succinyl  
3        substituents.

1        11. The peptide of claim 1, wherein the cleavage of the peptide by the enzyme yields at  
2        least 5 picomoles of cleaved peptide per minute per 200 picomoles of enzyme.

1        12. The peptide of claim 1, wherein the cleavage of the peptide in human serum yields at  
2        most 2.0 picomoles of cleaved peptide per minute.

1        13. A peptide of claim 1, further comprising an added substituent which renders the  
2        peptide water-soluble.

1        14. A peptide of claim 13, wherein the added substituent is a polysaccharide.

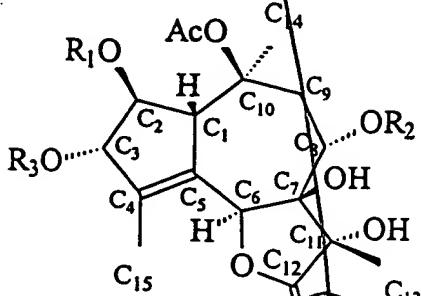
1        15. A peptide of claim 14, wherein the polysaccharide is selected from the group  
2        consisting of modified or unmodified dextran, cyclodextrin, and starch.

1        16. A peptide of claim 2, further comprising an antibody attached to the amino terminus  
2        of  $X_5$ , or  $X_4$  when  $X_5$  is 0.

1        17. A peptide composition comprising a plurality of peptides, each peptide comprising an  
2        amino acid sequence having a cleavage site specific for an enzyme having a proteolytic  
3        activity of prostate specific antigen, wherein each peptide has 20 or fewer amino acids.

1        18. A polynucleotide encoding the peptide of claim 1

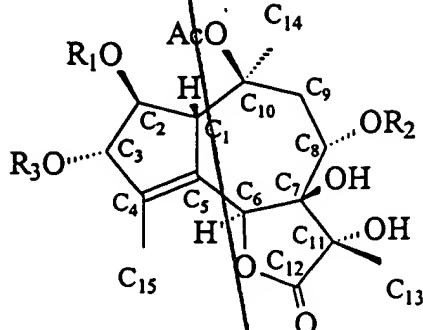
1       45. The thapsigargin derivative of claim 42, having the following structure



12       wherein R<sub>1</sub> is a primary amine-containing alkanoyl, alkenoyl, or arenoyl substituent, R<sub>2</sub> is  
13       an alkanoyl, alkenoyl, or arenoyl substituent, and R<sub>3</sub> is an alkanoyl or alkenoyl substituent.

1       46. The thapsigargin derivative of claim 45, wherein R<sub>1</sub> is selected from the group  
2       consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-  
3       substituted CO-(CH=CH)<sub>n1</sub>-(CH<sub>2</sub>)<sub>n2</sub>-Ar-NH<sub>2</sub>, CO-(CH<sub>2</sub>)<sub>n2</sub>-(CH=CH)<sub>n1</sub>-Ar-NH<sub>2</sub>, CO-(CH<sub>2</sub>)<sub>n2</sub>  
4       (CH=CH)<sub>n1</sub>-CO-NH-Ar-NH<sub>2</sub> and CO-(CH=CH)<sub>n1</sub>-(CH<sub>2</sub>)<sub>n2</sub>-CO-NH-Ar-NH<sub>2</sub>, wherein n1 and  
5       n2 are from 0 to 5, and Ar is any substituted or unsubstituted aryl group.

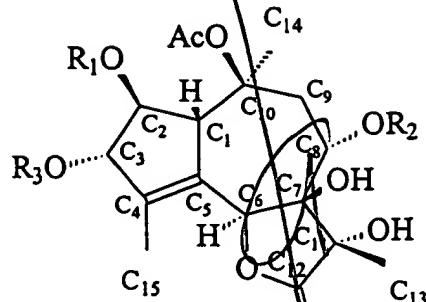
1           47. The thapsigargin derivative of claim 42, having the following structure



13       wherein R<sub>1</sub> is an alkanoyl, alkenoyl, or arenoyl substituent, R<sub>2</sub> is a primary amine-containing alkanoyl, alkenoyl, or arenoyl substituent, and R<sub>3</sub> is an alkanoyl or alkenoyl substituent.

1       48. The thapsigargin derivative of claim 47, wherein R<sub>2</sub> is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-substituted CO-(CH=CH)<sub>n1</sub>-(CH<sub>2</sub>)<sub>n2</sub>-Ar-NH<sub>2</sub>, CO-(CH<sub>2</sub>)<sub>n2</sub>-(CH=CH)<sub>n1</sub>-Ar-NH<sub>2</sub>, CO-(CH<sub>2</sub>)<sub>n2</sub>-(CH=CH)<sub>n1</sub>-CO-NH-Ar-NH<sub>2</sub> and CO-(CH=CH)<sub>n1</sub>-(CH<sub>2</sub>)<sub>n2</sub>CO-NH-Ar-NH<sub>2</sub>, wherein n1 and n2 are from 0 to 5, and Ar is any substituted or unsubstituted aryl group.

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15 49. The thapsigargin derivative of claim 48, having the following structure



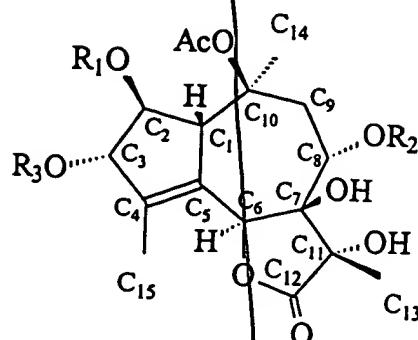
wherein R<sub>2</sub> is CO-CH=CH-Ph-p-NH<sub>2</sub>, wherein Ph-p-NH<sub>2</sub> is the para-aminophenyl substituent.

1       50. The thapsigargin derivative of claim 48, having the following structure

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15       wherein  $R_2$  is  $\text{CO}-\text{CH}_2-\text{CH}_2-\text{Ph}-\text{p-NH}_2$ , wherein  $\text{Ph-p-NH}_2$  is the para-aminophenyl  
16       substituent.

1       51. The thapsigargin derivative of claim 42, wherein the derivative has an  $\text{IC}_{50}$  toward ER  
2        $\text{Ca}^{2+}\text{-ATP-ase}$  of at most 500 nM.

1       52. The thapsigargin derivative of claim 51, wherein the derivative has an  $\text{IC}_{50}$  toward ER  
2        $\text{Ca}^{2+}\text{-ATP-ase}$  of at most 50 nM.

1       53. The thapsigargin derivative of claim 42, wherein the derivative has an  $\text{LC}_{50}$  toward  
2       PSA-producing tissue of at most 20  $\mu\text{M}$ .

1       54. The thapsigargin derivative of claim 53, wherein the derivative has an  $\text{LC}_{50}$  toward  
2       PSA-producing tissue of at most 2.0  $\mu\text{M}$ .

1           74. The method of claim 66, wherein the detectable label is a chemiluminescent label.

1           75. A method of selecting a prostate specific antigen activatable prodrug wherein the  
2           prodrug is substantially specific for target tissue comprising prostate specific antigen-  
3           producing cells, the method comprising:

- 4           a) linking a peptide of claim 1 to a therapeutic drug to produce a peptide-drug  
5           composition;
- 6           b) contacting the composition with cells of the target tissue;
- 7           c) contacting the composition with cells of a non-target tissue; and  
8           selecting complexes that are substantially toxic towards target tissue cells, but  
9           which are not substantially toxic towards non-target tissue cells.

1           76. A method of determining the activity of prostate specific antigen (PSA) in a  
2           sample containing PSA, the method comprising:

- 3           a) contacting the sample with a composition comprising a detectably labeled  
4           peptide of claim 1 for a period of time sufficient to allow cleavage of the peptide;
- 5           b) detecting the detectable label to yield a detection level;
- 6           c) comparing the detection level with a detection level obtained from contacting  
7           the detectably labeled peptide with a standard PSA sample.

1           77. A method of imaging PSA-producing tissue, the method comprising:

- 2           a) administering a peptide linked to a lipophilic imaging label to a subject having  
3           or suspected of having a PSA producing associated cell-proliferative disorder;
- 4           b) allowing a sufficient period of time to pass to allow cleavage of the peptide by  
5           PSA and to allow clearance of uncleaved peptide from the subject to provide a  
6           reliable imaging of the imaging label; and
- 7           c) imaging the subject.